08-01-16

Wrong enzymes used for WC1 and WC2 !

NEW CLONING

- Digest of
    WC1 with XbaI and Pst1
    WC2 with EcoRI and Spe1
    psB1C3 with XbaI and Pst1
    psB1C3 with EcoRI and Spe1

- Put on the incubator at 37°C during 1h

- Dephosphorylation of psB1C3
    Put on the incubator at 37°C during 1h

- Phosphatase inactivation
    At 80°C during 10 min

- Ligation of:
    WC1 in psB1C3
    WC2 in psB1C3
    Put on the fridge overnight

SET UP MINICULTURES

- Pick selected colony and add it on 6mL of LB with Chloramphenicol
    P2A in psB1C3 GFP (X3)
    NLP22 in psB1C3 (X2)
    DSIP in psB1C3 (X2)

- Put tubes on the shaking incubator at 37°C

MINIPREP

- Miniprep of:
    WC1 in psB1C3 (X1)
    pFRQ in psB1C3 (X1)
    NLP22 in psB1C3 (X4)
    DSIP in psB1C3 (X4)
    P2A GFP in psB1C3 (X3)
    NLP22 in psB1C3 (X2)
    DSIP in psB1C3 (X2)

PCR

- Amplification of:
  pRPS27 (with HF buffer)
  pRPS27 (with GC buffer)
  + HF control and GC control

Hybridation temperature used: 45°C
Elongation time used: 25sec

08-02-16

TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 containing pXBP1(gibson 07-28-16) (LB + Chloramphenicol)
    psB1C3 containing pUNC119 (gibson 07-28-16)
    psB1C3 containing WC1 (cloning 08-01-16)
    psB1C3 containing WC2(cloning 08-01-16)

- Agar plates put at 37°C

GEL MIGRATION

- Gel verification of amplification (pcr product of pRPS27 ; 08-01-16)
    Agarose gel: 1%
    100 mV, 30 min

=> No amplification observed

QUANTITATIVE ANALYSIS (Miniprep 08-01-16)

psB1C3 P2A GFP 1: 22,2 ng/µL
psB1C3 P2A GFP 2: 25,3 ng/µL
psB1C3 P2A GFP 3: 7,4 ng/µL
psB1C3 NLP22 1: 20,1 ng/µL
psB1C3 NLP22 2: 33,4 ng/µL
psB1C3 NLP22 3: 21,3 ng/µL
psB1C3 NLP22 4: 19,6 ng/µL
psB1C3 NLP22 5: 26,7 ng/µL
psB1C3 NLP22 6: 29,8 ng/µL
psB1C3 DSIP 1: 27,1 ng/µL
psB1C3 DSIP 2: 10,7 ng/µL
psB1C3 DSIP 3: 22,9 ng/µL
psB1C3 DSIP 4: 20,9 ng/µL
psB1C3 DSIP 5: 21,2 ng/µL
psB1C3 DSIP 6: 18,2 ng/µL
psB1C3 pFRQ 4: 20,9 ng/µL

GEL MIGRATION

- Gel verification of cloning
    Fisrt, the plasmid is digested with EcorI and PstI 1h at 37°C (+ control only digested with P)
    Agarose gel: 1%
    100 mV, 30 min

Gel Map:

2-log ladder
empty vector
psB1C3 P2A GFP 1
psB1C3 P2A GFP 2
psB1C3 pFRQ 1
psB1C3 pFRQ 2
psB1C3 NLP22 1
psB1C3 DSIP 1

=> GOOD:
    psB1C3 P2A GFP 2
    psB1C3 pFRQ 1
    psB1C3 pFRQ 2
    psB1C3 NLP22 1

08-03-16

GEL MIGRATION

- Gel verification of cloning
    Fisrt, the plasmid is digested with EcorI and PstI 1h at 37°C (+ control only digested with P)
    Agarose gel: 1%
    100 mV, 30 min

Gel Map:
2-log ladder
empty vector
psB1C3 NLP22 2
psB1C3 NLP22 3
psB1C3 NLP22 4
psB1C3 NLP22 5
psB1C3 NLP22 6

Gel Map:
2-log ladder
empty vector
psB1C3 DSIP 2
psB1C3 DSIP 3
psB1C3 DSIP 4
psB1C3 DSIP 5
psB1C3 DSIP 6

=> NOTHING !

CLONING

- Digest of
    psB1C3 with EcoRI and PstI
    pFRQ with EcoRI and SpeI
    NLP22 with XbaI and PstI
    psB1C3 GFP with EcoRI and SpeI

- Put on the incubator at 37°C during 1h

- Dephosphorylation of psB1C3 and psB1C3 GFP
    Put on the incubator at 37°C during 1h

- Phosphatase inactivation
    At 80°C during 10 min

- Ligation
    NLP22 in psB1C3
    pFRQ in psB1C3
    Put on the fridge overnight

CLONING

- Digest of
    psB1C3 with XbaI and PstI
    pUNC 119 with XbaI and PstI
    psB1C3 with EcoRI and PstI
    pXBP1 with EcoRI and PstI

- Put on the incubator at 37°C during 1h

- Dephosphorylation of psB1C3
    Put on the incubator at 37°C during 1h

- Phosphatase inactivation
    At 80°C during 10 min

- Ligation
    pUNC119 in psB1C3
    pXBP1 in psB1C3
    Put on the fridge overnight

PCR

- Amplification of:
    WC1
    WC2
    + Control

Hybridation temperature used: 63°C
Elongation time used: 2min 15sec

PCR

- Amplification of:
    pRPS27
    + Control

Hybridation temperature used: XX°C
Elongation time used: XXsec

08-04-16

GEL MIGRATION

- Gel verification for an amplification of WC1 and WC2 (pcr product ; 08-01-16)
    Agarose gel: 1%
    100 mV, 30 min

=> No amplification observed

- Gel verification for NLP22 inserted in psB1C3
    Fisrt, the plasmid is digested with EcorI and PstI (1h at 37°C)
    Agarose gel: 1%
    100 mV, 30 min

=> NOTHING

SEQUENCING

- Some miniprep tubes are send to a sequencing platform to verify the efficacity of cloning
    psB1C3 P2A GFP 1
    psB1C3 pFRQ 2
    psB1C3 NLP22 3

=> Sequencing came back: GOOD !!

TRANSFORMATION

- Transformation in DH5α of:
    pFB001 containing pRPS27 (LB + Ampicilline)
    psB1C3 containing pUNC119 (LB + Chloramphenicol)
    psB1C3 containing pXBP1
    psB1C3 containing DSIP

- Agar plates put at 37°C

CLONING

- Cloning of DSIP in psB1C3 with:
    Digest tubes kept on the fridge (date)
    Enzymes used: EcorI and PstI

- Ligation
    DSIP in psB1C3
    Put on the fridge overnight

08-05-16

TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 containing DSIP (LB + Chloramphenicol)

- Agar plates put at 37°C

PCR

- Amplification of:
    pRPS27
    + Control

Hybridation temperature used: XX°C
Elongation time used: XXsec

08-08-16

GEL MIGRATION

- Gel verification of pRPS27 amplification (done the 08-05-16)
    Agarose gel: 1%
    100 mV, 30 min

=> No amplification observed

SET UP MINICULTURES

- Pick selected colony and put it in 6mL of LB with chloramphenicol
    psB1C3 containing DSIP (X6)
    psB1C3 containing pFRQ GFP (X3)
    psB1C3 containing pFRQ NLP22 (X3)
    psB1C3 containing pXBP1 (X3)
    psB1C3 containing pUNC119 (X3)

- Put at 37°C on the shaking incubator

CLONING

- Cloning of NLP22 in psB1C3 with:
    Digest tubes kept on the fridge
    Ligation tube kept on the fridge
    Enzymes used: EcoRI and PstI

TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 containing NLP22 (LB + Chloramphenicol)

- Put agar plates at 37°C

PCR

- Amplification of:
    pRPS27
    + Control

Hybridation temperature used: 61°C
Elongation time used: 25 sec

08-09-16

MINIPREP

- Miniprep kit used on 08-08-16 tubes:
    - psB1C3 containing DSIP (X6)
    - psB1C3 containing pFRQ GFP (X3)
    - psB1C3 containing pFRQ NLP22 (X3)
    - psB1C3 containing pXBP1 (X3)
    - psB1C3 containing pUNC119 (X3)

QUANTITATIVE ANALYSIS

psB1C3 DSIP 1: 66,2 ng/µL
psB1C3 DSIP 2: 73,4 ng/µL
psB1C3 DSIP 3: 66,6 ng/µL
psB1C3 DSIP 4: 44,0 ng/µL
psB1C3 DSIP 5: 53,7 ng/µL
psB1C3 DSIP 6: 61,4 ng/µL
psB1C3 pFRQ GFP 1: 9,8 ng/µL
psB1C3 pFRQ GFP 2: 5,7 ng/µL
psB1C3 pFRQ GFP 3: 8,3 ng/µL
psB1C3 pFRQ NLP22 1: 39,1 ng/µL
psB1C3 pFRQ NLP22 2: 66,3 ng/µL
psB1C3 pFRQ NLP22 3: 53,7 ng/µL
psB1C3 pXBP1 1: 45,7 ng/µL
psB1C3 pXBP1 2: 90,0 ng/µL
psB1C3 pXBP1 3: 62,4 ng/µL
psB1C3 pUNC119 1: 59,1 ng/µL
psB1C3 pUNC119 2: 82,6 ng/µL
psB1C3 pUNC119 3: 45,7 ng/µL

PCR

- Amplification of:
    - pU6 (pMB70)
    - pU6 (gDNA of C.elegans)
    - + Control

Hybridation temperature used: 65°C
Elongation time used: 10 sec

GEL MIGRATION

- Gel verification of some miniprep tubes:
    Fisrt, plasmids are digested with EcoRI and PstI (1h at 37°C)
    Agarose gel: 1%
    100 mV, 30 min

Gel Map:

Ladder
psB1C3 DSIP 1
psB1C3 DSIP 2
psB1C3 DSIP 3
psB1C3 DSIP 4
psB1C3 pFRQ GFP 1
psB1C3 pFRQ GFP 2
psB1C3 pFRQ GFP 3
psB1C3 pFRQ NLP22 1
psB1C3 pFRQ NLP22 1
psB1C3 pFRQ NLP22 1

=> GOOD:
    psB1C3 pFRQ NLP22 2

- Gel verification of pU6 amplification
    Agarose gel: 1%
    100 mV, 30 min

Gel Map:

Ladder
Control
pU6 (pMB70)
pU6 (C. Elegans's gDNA)

=> Amplication of pU6 (gDNA)

TRANSFORMATION

- Transformation in DH5α of:
    pMB70 containing pU6 (LB + Kanamycine)

- Agar plate put at 37°C

08-10-16

CLONING

- Cloning done with frozen miniprep tubes
    - psB1C3 containing DSIP
    - psB1C3 containing NLP22
    - psB1C3 containing pFRQ
    - psB1C3 containing pU6

TRANSFORMATION

- Transformation in DH5α of:
    - psB1C3 containing DSIP (LB + Choramphenicol)
    - psB1C3 containing NLP22
    - psB1C3 containing pU6

- Agar plate put at 37°C

GEL MIGRATION

- Gel verification of some miniprep tubes (08-09-16):
    - Fisrt, plasmids are digested with EcoRI and PstI (1h at 37°C)
    - Agarose gel: 1%
    - 100 mV, 30 min

Gel Map:

Ladder
psB1C3 DSIP 5
psB1C3 DSIP 6
psB1C3 pXBP1 1
psB1C3 pXBP1 2
psB1C3 pXBP1 3
psB1C3 pUNC119 1
psB1C3 pUNC119 2
psB1C3 pUNC119 3

=> GOOD:
- psB1C3 DSIP 5
- psB1C3 DSIP 6
- psB1C3 pXBP1 2
- psB1C3 pXBP1 3

SET UP MINICULTURES

- Pick selected colony and put it in 6mL of LB with chloramphenicol
    psB1C3 pFRQ GFP (X1)
    psB1C3 NLP22 (X2)

- Put on the shaking incubator at 37°C

PCR

- Amplification on C.elegans's gDNA of:
    pRPS27
    pmyo 1
    pmyo 2
    pUNC119
    pXBP1

- Amplification on Neurospora terrasperma's gDNA of:
    WC1
    WC2
    + control

Hybridation temperature used: 65°C
Elongation time used: 2 min 15 sec

GEL MIGRATION

- Gel verification of amplifications
    Agarose gel: 1%
    100 mV, 30 min

Gel Map:

Ladder
Control
pRPS27
pRPS27
pXBP1
pUNC119
WC1
WC2
pmyo2
pmyo3

=> GOOD:
    pXBP1
    WC1
    WC2

08-11-16

MINIPREP

- Miniprep kit used on 08-10-16 tubes:
    psB1C3 NLP22 1
    psB1C3 NLP22 2
    psB1C3 pFRQ GFP 4

QUANTITATIVE ANALYSIS

psB1C3 NLP22 1: 103,7 ng/µL
psB1C3 NLP22 2: 71,4 ng/µL
psB1C3 pFRQ GFP 4: 11,7 ng/µL

GEL MIGRATION

- Gel verification of miniprep tubes:
    Fisrt, plasmids are digested with EcoRI and PstI (1h at 37°C)
    Agarose gel: 1%
    100 mV, 30 min

Gel Map:

Ladder
psB1C3 NLP22 1
psB1C3 NLP22 2
psB1C3 pFRQ GFP 4
control (empty psB1C3)
psB1C3 pFRQ 5
psB1C3 pFRQ NLP22 3
psB1C3 pUNC119

=> Weird results

SET UP MINICULTURES

- Pick selected colony and put it in 6mL of LB with chloramphenicol
    psB1C3 containing pFRQ
    pMB70 containing pU6

- Put on the shaking incubator at 37°C

SEQUENCING

- Some miniprep tubes are send to a sequencing platform to verify the efficacity of cloning:
    psB1C3 DSIP 6
    psB1C3 pFRQ NLP22 2
    psB1C3 pXBP1 2

=> Sequencing came back: Only psB1C3 pXBP1 is good

CLONING

- Cloning of pU6 in psB1C3 GFP
    pU6 cut with EcorI and SpeI
    psB1C3 cut with XbaI and PstI

- Put on the incubator at 37°C during 1h

- Dephosphorylation of psB1C3 GFP
    Put on the incubator at 37°C during 1h

- Phosphatase inactivation
    At 80°C during 10min

- Ligation
    Put 1h at room temperature

TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 GFP containing pU6 (LB + Choramphenicol)

- Put on the incubator at 37°C

CLONING

- Ligation of pFRQ in psB1C3 GFP (with digested tubes kept on the fridges 07-28-16)
    Put 1h at room temperature

TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 GFP containing pFRQ (LB + Choramphenicol)

- Put on the incubator at 37°C

PCR

- Amplification of:
    pRPS27
    + Control

Hybridation temperature used: 61°C
Elongation time used: 35 sec

GEL MIGRATION

- Gel verification of amplification
    Agarose gel: 1%
    100 mV, 30 min

=> No amplification observed

PCR

- Amplification of:
    pmyo2
    pmyo3
    pUNC119
    + Control

Hybridation temperature used: 65°C
Elongation time used: 1 min 10 sec

GEL MIGRATION

- Gel verification of amplification
    Agarose gel: 1%
    100 mV, 30 min

Gel Map:

Ladder
Control
pUNC119
pmyo2
pmyo3

=> Successful amplification of pmyo2

SET UP MINICULTURES

- Pick selected colony of agar plates kept on the fridge and put it on 6mL of LB + Chloramphenicol
    psB1C3 containing DSIP (X2)
    psB1C3 containing pFRQ and NLP22 (X2)
    psB1C3 containing pXBP1 (X2)

- Put on the shaking incubator at 37°C

CLONING

WC1 cut with XbaI and PstI
WC2 cut with EcoRI and SpeI

NLP22 cut with EcoRI and Pst1
pFRQ cut with EcoRI and Pst1
DSIP cut with EcoRI and Pst1

DSIP cut with EcoRI and XbaI
psB1C3 P2A GFP cut with EcoRI and XbaI

- Put on the incubator at 37°C during 1h

- Dephosphorylation of psB1C3 P2A GFP
    Put on the incubator at 37°C during 1h

- Phosphatase inactivation
    At 80°C during 10min

- Ligation
    Put on the fridge (4°C) overnight

08-12-16

TRANSFORMATION

- Transformation in DH5α of:
    - psB1C3 containing WC1 (LB + Chloramphenicol)
    - psB1C3 containing WC2
    - psB1C3 containing NLP22
    - psB1C3 containing pFRQ
    - psB1C3 containing DSIP
    - psB1C3 containing DSIP P2A GFP

- Put agar plates on the incubator at 37°C

SET UP MINICULTURES

- Pick selected colony of agar plates kept on the fridge and put it on 6mL of LB + Chloramphenicol
    - psB1C3 containing pU6 (X1)
    - psB1C3 containing DSIP (X2)
    - psB1C3 containing NLP22 (X3)

- Put on the shaking incubator at 37°C

MINIPREP

- Miniprep kit used on 08-10-16 tubes:
    - pMB70 pU6 1
    - psB1C3 pFRQ A

- Miniprep kit used on 08-11-16 tube:
    - psB1C3 DSIP A1
    - psB1C3 DSIP B1
    - psB1C3 pFRQ NLP22 A
    - psB1C3 pFRQ NLP22 B
    - psB1C3 pXBP1 A
    - psB1C3 pXBP1 B

QUANTITATIVE ANALYSIS

pMB70 pU6 1: 79,2 ng/µL
psB1C3 pFRQ A: 68,8 ng/µL
psB1C3 DSIP A1: 28,9 ng/µL
psB1C3 DSIP B1: 46,5 ng/µL
psB1C3 pFRQ NLP22 A: 33,1 ng/µL
psB1C3 pFRQ NLP22 B: 53,2 ng/µL
psB1C3 pXBP1 A: 47,2 ng/µL
psB1C3 pXBP1 B: 57,3 ng/µL

CLONING

- Cloning of pU6 in psB1C3 containing DSIP (psB1C3 DSIP 5)
    pU6 cut with EcorI and XbaI
    psB1C3 cut with EcorI and XbaI
    Put on the incubator at 37°C during 1h

- Dephosphorylation of psB1C3
    Put on the incubator at 37°C during 1h

- Phosphatase inactivation
    At 80°C during 10min

- Ligation
    Put on the fridge (4°C) overnight

08-15-16

TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 containing pU6 DSIP (LB + Chloramphenicol)

- Put agar plates on the incubator at 37°C

MINIPREP

- Miniprep kit used on 08-12-16 tubes:
    psB1C3 containing pU6 1
    psB1C3 containing DSIP 1
    psB1C3 containing DSIP 2
    psB1C3 containing NLP22 1
    psB1C3 containing NLP22 2
    psB1C3 containing NLP22 3

08-16-16

PCR

- Amplification of:
    pRPS27
    pUNC119
    pmyo3
    + Control

Hybridation temperature used: 68°C
Elongation time used: 1 min 10 sec

GEL MIGRATION

- Gel verification of amplification
    Agarose gel: 1%
    100 mV, 30 min

=> No amplification observed

- Gel verification of cloning (08-15-16 miniprep tubes)
    Fisrt, the plasmid is digested with EcorI and PstI 1h at 37°C (+ control only digested with P)
    Agarose gel: 1%
    100 mV, 30 min

Gel Map:

Ladder
Control
psB1C3 NLP22 A
psB1C3 NLP22 B
psB1C3 NLP22 C
psB1C3 DSIP A
psB1C3 DSIP B
psB1C3 pU6

=> NOTHING

SET UP MINICULTURES

- Pick selected colony of agar plates and put it on 6mL of LB + Chloramphenicol
    - psB1C3 containing DSIP 2A (X4)
    - psB1C3 containing pFRQ (X2)
    - psB1C3 containing NLP22 (X4)
    - psB1C3 containing DSIP (X2)
    - psB1C3 containing pU6 DSIP (X2)
    - psB1C3 containing WC1 (X2)
    - psB1C3 containing WC2 (X1)

- Put on the shaking incubator at 37°C

08-17-16

MINIPREP

- Miniprep kit used on 08-16-16 tubes:
    - psB1C3 DSIP 2A GFP 1
    - psB1C3 DSIP 2A GFP 2
    - psB1C3 DSIP 2A GFP 3
    - psB1C3 DSIP 2A GFP 4
    - psB1C3 pFRQ B1
    - psB1C3 pFRQ B2
    - psB1C3 NLP22 B1
    - psB1C3 NLP22 B2
    - psB1C3 NLP22 B3
    - psB1C3 NLP22 B4
    - psB1C3 DSIP 1
    - psB1C3 DSIP 2
    - psB1C3 pU6 DSIP 1
    - psB1C3 pU6 DSIP 2
    - psB1C3 WC1 1
    - psB1C3 WC1 2
    - psB1C3 WC2 1

GEL MIGRATION

- Gel verification of miniprep tubes
    - Fisrt, the plasmid is digested with EcorI and PstI 1h at 37°C
    - Not for WC1 and WC2 (others enzymes used)
    - + control only digested with P

    - Agarose gel: 1%
    - 100 mV, 30 min

Gel Map:

Ladder
Control
psB1C3 NLP22 1
psB1C3 NLP22 2
psB1C3 NLP22 3
psB1C3 NLP22 4
psB1C3 DSIP 2A GFP 1
psB1C3 DSIP 2A GFP 2
psB1C3 DSIP 2A GFP 3
psB1C3 DSIP 2A GFP 4
psB1C3 pFRQ B1
psB1C3 pFRQ B2

=> Weird results

Gel Map:

Ladder
Control
psB1C3 WC1 1
psB1C3 WC1 2
psB1C3 WC2 1
psB1C3 pU6
psB1C3 DSIP 1
psB1C3 DSIP 2
psB1C3 pU6 DSIP 1
psB1C3 pU6 DSIP 2

=> Weird results

SET UP MINICULTURES

- Pick selected colony of agar plates and put it on 6mL of LB + Chloramphenicol
  psB1C3 containing WC1 (X2)
  psB1C3 containing WC2 (X2)

- Put on the shaking incubator at 37°C

CLONING

- Cloning of pU6 in psB1C3
  pU6 cut with EcorI and PstI
  pU6 cut with EcorI and SpeI
  psB1C3 cut with EcorI and PstI
  psB1C3 GFP cut with EcoRI and XbaI

- Put on the incubator at 37°C during 1h

- Dephosphorylation of psB1C3
  Put on the incubator at 37°C during 1h

- Phosphatase inactivation
  At 80°C during 10min

- Ligation
  Put 1h at room temperature

TRANSFORMATION

- Transformation in DH5α of:
  psB1C3 containing pU6 (LB + Chloramphenicol)
  psB1C3 GFP containing pU6

- Agar plates put at 37°C

PCR

- Amplification of:
    pRPS27 (pFB001)
    pRPS27 (gDNA)
    pmyo3
    pUNC119
    + Control

Hybridation temperature used: 63°C
Elongation time used: 1min 10sec

GEL MIGRATION

- Gel verification of amplification
    Agarose gel: 1%
    100 mV, 30 min

=> No amplification observed

PCR

- Amplification of:
    pU6 (pMB70)
    pU6 (gDNA)
    + Control

Hybridation temperature used: 65°C
Elongation time used: 10sec

GEL MIGRATION

- Gel verification of amplification
    Agarose gel: 1%
    100 mV, 30 min

Gel Map:

Ladder
Control
pU6 (pMB70)
pU6 (gDNA)

=> Successful amplification of pU6 (pMB70)

CLONING

- Cloning of:
    pU6 cut with EcorI and SpeI
    psB1C3 GFP cut with EcoRI and XbaI
    psB1C3 pFRQ cut with SpeI and PstI
    psB1C3 GFP cut with XbaI and PstI
    NLP22 cut with XbaI and PstI

- Put on the incubator at 37°C during 1h

- Dephosphorylation of psB1C3 (3 tubes)
    Put on the incubator at 37°C during 1h

- Phosphatase inactivation
    At 80°C during 10min

- Ligation
    Put on the fridge overnight

08-18-16

TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 containing pU6 and GFP (LB + Chloramphenicol)
    psB1C3 containing pFRQ and NLP22
    psB1C3 containing pFRQ and GFP

- Agar plates put at 37°C

CLONING

- Cloning of:
    DSIP cut with EcorI and PstI
    NLP22 cut with EcoRI and PstI
    psB1C3 cut with EcoRI and PstI
    psB1C3 GFP cut with EcoRI and XbaI
    P2A cut with EcoRI and SpeI

- Put on the incubator at 37°C during 1h

- Dephosphorylation of psB1C3 (3 tubes)
    Put on the incubator at 37°C during 1h

- Phosphatase inactivation
    At 80°C during 10min

- Ligation
    Put 1h at room temperature

TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 containing DSIP (LB + Chloramphenicol)
    psB1C3 containing NLP22
    psB1C3 containing P2A and GFP

- Agar plates put at 37°C

SET UP MINICULTURES

- Pick selected colony and add it on 6mL of LB with Chloramphenicol
    DSIP in psB1C3 (X2)
    NLP22 in psB1C3 (X2)
    pFRQ in psB1C3 (X2)
    pU6 DSIP in psB1C3 (X2)
    DSIP P2A GFP in psB1C3 (X2)

- Put tubes on the shaking incubator at 37°C

CLONING

- Cloning of:
    pU6 cut with EcorI and SpeI
    DSIP cut with EcoRI and XbaI
    pFRQ cut with EcoRI and SpeI
    NLP22 cut with EcoRI and XbaI

- Put on the incubator at 37°C during 1h

- Dephosphorylation of IDT plasmid (2 tubes)
    Put on the incubator at 37°C during 1h

- Phosphatase inactivation
    At 80°C during 10min

- Ligation
    Put on the fridge overnight

PCR

- Amplification of:
    pRPS27 (pFB001)
    pRPS27 (gDNA)
    + Control

Hybridation temperature used: 61°C
Elongation time used: 35sec

08-19-16

TRANSFORMATION

- Transformation in DH5α of:
    IDT plasmid containing pU6 and DSIP (LB + Ampicillin)
    IDT plasmid containing pFRQ and NLP22

- Agar plates put at 37°C

CLONING

- Cloning of pmyo2:
    pmyo2 cut with XbaI and SpeI
    psB1C3 cut with XbaI and SpeI

- Put on the incubator at 37°C during 1h

- Dephosphorylation of IDT plasmid (2 tubes)
    Put on the incubator at 37°C during 1h

- Phosphatase inactivation
    At 80°C during 10min

- Ligation
    Put on the fridge overnight

MINIPREP

- Miniprep of:
    WC1 in psB1C3 (X1)
    WC2 in psB1C3 (X1)

GEL MIGRATION

- Gel verification of amplification (08-18-16) and miniprep tubes
    Fisrt, the plasmid is digested 1h at 37°C
    Agarose gel: 1%
    100 mV, 30 min

Gel Map:

Ladder
Control
pRPS27 (plasmid)
pRPS27 (gDNA)
psB1C3 WC1
psB1C3 WC2

=> GOOD:
    psB1C3 WC2

PCR

- Amplification of:
    pmyo3
    pUNC119
    + Control

Hybridation temperature used: 63°C
Elongation time used: 1min 05sec

GEL MIGRATION

- Gel verification of amplification
    Agarose gel: 1%
    100 mV, 30 min

=> No amplification obesrved

SITE DIRECTED MUTAGENESIS

- Amplification with specific primers allowing mutagenesis of:
    psB1C3 pXBP1 (on XbaI site)
    psB1C3 WC2
    + control

Hybridation temperature used: 50°C
Elongation time used: 1min 05sec

- Put tubes on the fridge

MINIPREP

- Miniprep of:
    - psB1C3 WC1 A
    - psB1C3 WC2 A
    - psB1C3 NLP22 A
    - psB1C3 NLP22 B
    - psB1C3 pU6 DSIP A
    - psB1C3 pU6 DSIP B
    - psB1C3 DSIP A
    - psB1C3 DSIP P2A GFP A
    - psB1C3 pFRQ A
    - psB1C3 pFRQ B

SET UP MINICULTURES

- Pick selected colony and add it on 6mL of LB with Chloramphenicol
    - P2A in psB1C3 GFP (X2)
    - pFRQ NLP22 in psB1C3 (X2)
    - DSIP in psB1C3 (X2)
    - NLP22 in psB1C3 (X2)
    - pFRQ in psB1C3 GFP (X1)

- Put tubes on the shaking incubator at 37°C

PCR

- Amplification of:
    - pRPS27 (pFB001)
    - pRPS27 (gDNA)
    - + Control

Hybridation temperature used: XX°C
Elongation time used: XXsec

08-22-16

END OF SITE DIRECTEDMUTAGENESIS

- Add 1µL of DnpI on:
    mutated pXBP1
    mutated WC2

- Put at 37°C during 1h

TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 containing mutated WC2 (LB + Chloramphenicol)
    psB1C3 containing mutated pXBP1
    psB1C3 containing pmyo2

- Agar plates put at 37°C

GEL MIGRATION

- Gel verification of amplifications and miniprep tubes (08-19-16)
    Fisrt, the plasmid is digested 1h at 37°C
    Agarose gel: 1%
    100 mV, 30 min

Gel Map:

Ladder
psB1C3 WC1
psB1C3 WC2
Control
pRPS27 (gDNA)
pRPS27 (pFB001)

=> GOOD:
    psB1C3 WC1
    psB1C3 WC2

SET UP MINICULTURES

- Pick selected colony and add it on 6mL of LB with Ampicillin
    IDT plasmid containing pU6 DSIP (X3)
    IDT plasmid containing pFRQ NLP22 (X3)

- Put tubes on the shaking incubator at 37°C

GEL MIGRATION

- Gel verification of miniprep tubes
    Fisrt, the plasmid is digested 1h at 37°C
    Agarose gel: 1%
    100 mV, 30 min

Gel Map:

Ladder
psB1C3 NLP22 A
psB1C3 NLP22 B
Control
psB1C3 pFRQ A
psB1C3 pFRQ B
psB1C3 pU6 DSIP A
psB1C3 pU6 DSIP B
psB1C3 DSIP A
psB1C3 pFRQ GFP A

=> GOOD:
    psB1C3 NLP22 B
    psB1C3 pFRQ A
    psB1C3 pFRQ GFP A

SITE DIRECTED MUTAGENESIS

- Amplification with specific primers allowing mutagenesis of:
    psB1C3 WC1
    + control

Hybridation temperature used: 55°C
Elongation time used: 2min 10sec

- Add 1µL of DnpI on:
    mutated WC1

- Put at 37°C during 1h

TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 containing mutated WC1 (LB + Chloramphenicol)

- Agar plates put at 37°C

08-23-16

SEQUENCING

- Some miniprep tubes are send to a sequencing platform to verify the efficacity of cloning
    psB1C3 NLP22 B
    psB1C3 pFRQ GFP A
    psB1C3 pFRQ NLP22 A

=> Sequencing came back: psB1C3 pFRQ GFP A = GOOD !!

TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 containing pU6 GFP (LB + Chloramphenicol)
    psB1C3 containing pU6

- Agar plates put at 37°C

CLONING

- Digest of
    pU6 with EcoRI and SpeI
    psB1C3 GFP with EcoRI and XbaI

- Put on the incubator at 37°C during 1h

- Dephosphorylation of psB1C3 GFP
    Put on the incubator at 37°C during 1h

- Phosphatase inactivation
    At 80°C during 10 min

- Ligation
    NLP22 in psB1C3
    pFRQ in psB1C3
    Put 1h at room temperature

TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 containing pU6 (LB + Chloramphenicol)
    psB1C3 containing pU6 and GFP

- Agar plates put at 37°C

PCR

- Amplification of:
    pUNC119
    pmyo3
    + control

Hybridation temperature used: 60°C
Elongation time used: 1min 10sec

GEL MIGRATION

- Gel verification of amplifications
    Agarose gel: 1%
    100 mV, 30 min

=> No amplification observed

MINIPREP

- Miniprep of:
    pIDT pU6 DSIP 1
    pIDT pFRQ NLP22 1

GEL MIGRATION

- Gel verification of miniprep tubes
    Agarose gel: 1%
    100 mV, 30 min

=> Nothing

PCR

- Amplification of:
    pUNC119
    pmyo3
    + control

Hybridation temperature used: 55°C
Elongation time used: 1min 10sec

08-24-16

GEL MIGRATION

- Gel verification of amplifications (08-23-16)
    Agarose gel: 1%
    100 mV, 30 min

=> No amplification observed

SET UP MINICULTURES

- Pick selected colony and add it on 6mL of LB with Chloramphenicol
    pmyo2 in psB1C3 (XWC22)
    mutated WC1 in psB1C3 (X2)
    mutated WC2 in psB1C3 (X2)
    mutated pXBP1 in psB1C3 (X2)

- Put tubes on the shaking incubator at 37°C

MINIPREP

- Miniprep of:
    pU6 DSIP in pIDT
    pFRQ NLP22 in pIDT
    pU6 DSIP in psB1C3
    NLP22 in psB1C3

GEL MIGRATION

- Gel verification of cloning
    Fisrt, the plasmid is digested with EcorI and PstI 1h at 37°C (+ control only digested with P)
    Agarose gel: 1%
    100 mV, 30 min

Gel Map:
2-log ladder
empty vector
psIDT pU6 DSIP
psIDT pFRQ NLP22
psB1C3 pU6 DSIP
psB1C3 NLP22

=> Nothing

PCR

- Amplification of:
    - pUNC119
    - pmyo3
    - + control

Hybridation temperature used: 65°C
Elongation time used: 1min 10sec

08-25-16

SET UP MINICULTURES

- Pick selected colony and add it on 6mL of LB with Chloramphenicol
    pU6 in psB1C3 GFP (X1)
    pFRQ NLP22 in psB1C3 (X2)
    pFRQ GFP in psB1C3 (X1)
    DSIP in psB1C3 (X2)
    NLP22 in psB1C3 (X2)

- Put tubes on the shaking incubator at 37°C

GEL MIGRATION

- Gel verification of amplifications and miniprep tubes (08-24-16)
    Fisrt, the plasmid is digested with EcorI and PstI 1h at 37°C (+ control only digested with P)
    Agarose gel: 1%
    100 mV, 30 min

- Gel map:
2-log Ladder
Control
pmyo2
pUNC119
pIDT pU6 DSIP
pIDT pFRQ NLP22
psB1C3 pU6 DSIP

=> GOOD:
    pIDT pU6 DSIP
    pIDT pFRQ NLP22

TRANSFORMATION

- Transformation in DH5α of:
    psB1C3 containing pU6 and DSIP (LB + Chloramphenicol)

- Agar plate put at 37°C

MINIPREP

- Miniprep of:
    psB1C3 pU6 1
    psB1C3 DSIP A
    pIDT pU6 DSIP A
    pIDT pFRQ NLP22 A
    psB1C3 mutated pXBP1 1
    psB1C3 mutated WC1 1
    psB1C3 mutated WC2 1
    psB1C3 pmyo2 1

GEL MIGRATION

- Gel verification of miniprep tubes
    Fisrt, the plasmid is digested with EcorI and PstI 1h at 37°C (+ control only digested with P)
    Agarose gel: 1%
    100 mV, 30 min

- Gel map:
2-log Ladder
Control
psB1C3 pU6 1
psB1C3 DSIP A
pIDT pU6 DSIP A
pIDT pFRQ NLP22 A
psB1C3 mutated pXBP1 1
psB1C3 mutated WC1 1
psB1C3 mutated WC2 1
psB1C3 pmyo2 1

=> GOOD:
    psB1C3 mutated WC1 1
    psB1C3 mutated WC2 1
    psB1C3 mutated pXBP1 1

QUANTITATIVE ANALYSIS

psB1C3 pU6: 333,5 ng/µL
pIDT pU6 DSIP: 500,8 ng/µL
pIDT pFRQ NLP22: 240 ng/µL

PCR

- Amplification of:
    pUNC119
    + control
Hybridation temperature used: 63°C
Elongation time used: 1min 10sec

08-26-16

CLONING

- Digest of
    - mutated WC1 with EcoRI and XbaI
    - mutated WC2 with EcoRI and XbaI
    - pU6 with EcoRI and SpeI
    - psB1C3 GFP with EcoRI and XbaI
    - pFRQ with SpeI and PstI (pIDT)
    - NLP22 with XbaI and PstI (pIDT)
    - pFRQ with SpeI and PstI

- Put on the incubator at 37°C during 1h

- Dephosphorylation
    - Put on the incubator at 37°C during 1h

- Phosphatase inactivation
    - At 80°C during 10 min

- Ligation
    - pU6 and mutated WC1 in psB1C3
    - pU6 and mutated WC2 in psB1C3
    - pU6 and GFP in psB1C3
    - pFRQ and NLP22 in pIDT
    - pFRQ and NLP22 in psB1C3
    - Put 1h at room temperature

TRANSFORMATION

- Transformation in DH5α
- Agar plates put at 37°C

SITE DIRECTED MUTAGENESIS

- Amplification with specific primers allowing mutagenesis of:
    - psB1C3 pXBP1
    - + control

Hybridation temperature used: 50°C
Elongation time used: 1min 05sec

- Add 1µL of DnpI on:
    - mutated pXBP1

- Put at 37°C during 1h

TRANSFORMATION

- Transformation in DH5α of:
  psB1C3 containing mutated pXBP1 (LB + Chloramphenicol)

- Agar plates put at 37°C

WORM INJECTION

- Injection of pU6 DSIP in several worms with an other plasmid containing DsRed

QUANTITATIVE ANALYSIS

psB1C3 mutated WC1: 59,2 ng/µL
psB1C3 mutated WC2: 75,3 ng/µL
psB1C3 mutated pXBP1: 126,2 ng/µL